Neuroscience 201A Exam, 28 October 2013

This is a four hour exam (9:10 AM – 1:10 PM).

You can take the exam electronically, on paper, or by a combination of the two. If you take any part of the exam electronically, please confirm that I have received your electronic file by the end of the exam.

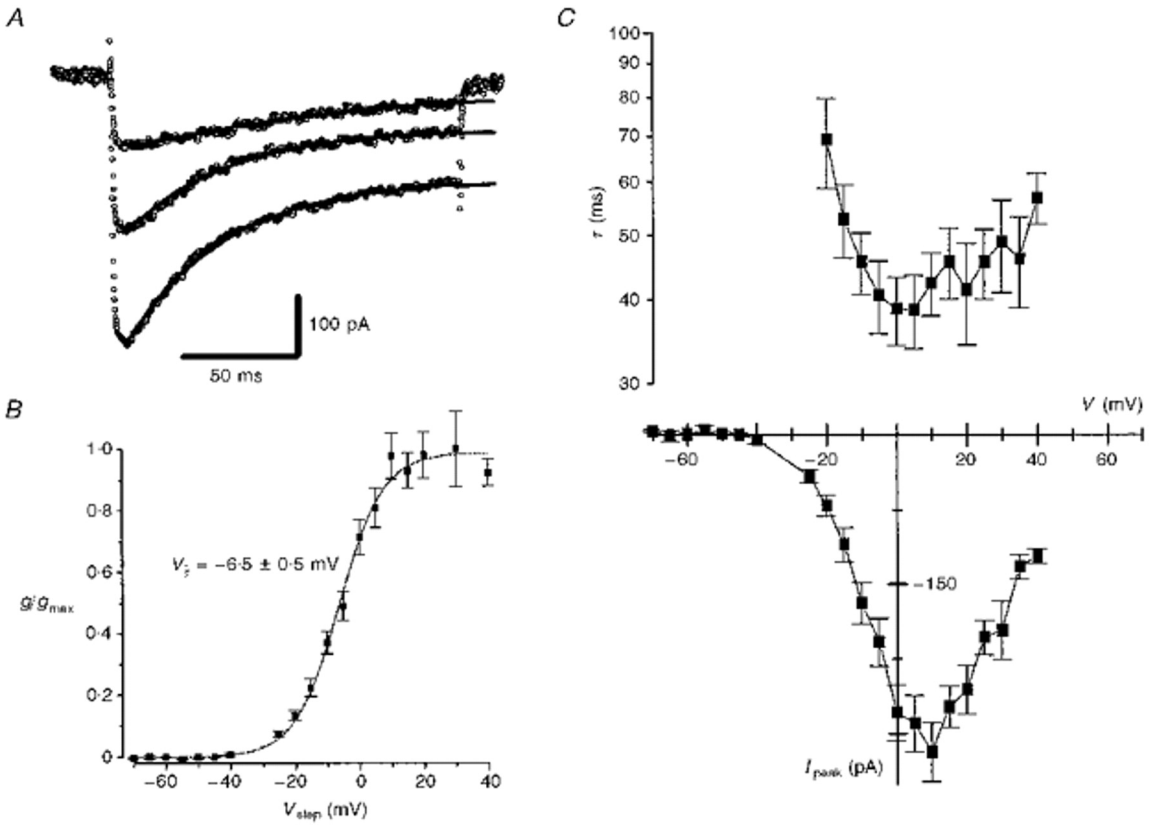
With the exception of the first question, you are to do your own work.

This is an open-book exam. Any materials that you have on your laptop or "in the cloud" that you have used in preparation for this exam, or that are available through the NS 201A web site, are available for you to use. You may not use other resources, e.g., PubMed, Wikipedia, etc. To finish the exam on time, you will need to use the "structure of understanding" about this material that you had built as of 9 AM today.

Question #1: 20 minutes (8 pts)

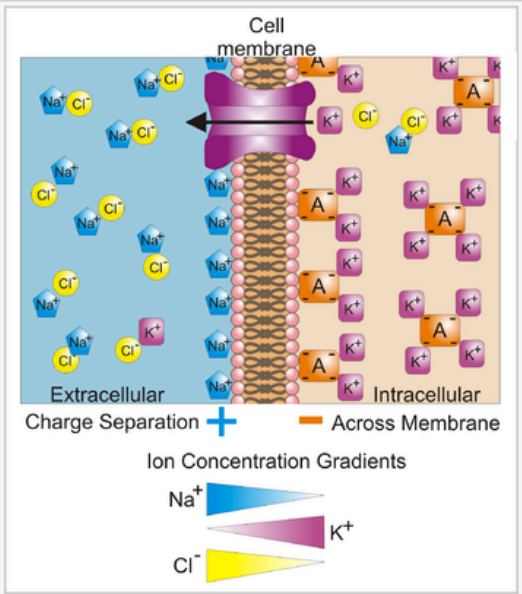
With increasing frequency, you will be learning from one another. Indeed, it's already happening in this course. To recognize and celebrate this transition, the first question in this exam is to be resolved by you communally. Talk through this problem as a group. Consider how best to respond to the questions. Hear everyone out (as a cerebral engine, you will most effective if you consider the most diverse set of inputs). Use the board. Once you are agreed how to respond, erase what you have on the board and write your responses individually without communicating with one another. Once you start writing, come get me and I will give you the rest of the exam. You are responsible for managing your time. This shouldn't take you more than about 25 minutes.

Question #1 starts on page 2



The figure above was obtained from a paper on calcium channels expressed by dentate granule cells. The whole-cell Ca^{2+} currents in (A) were measured in response to voltage steps from a holding potential of –60 mV to test potentials of –10, 0 , and + 10 mV. The external solution contained normal saline (2 mM Ca^{2+}) plus tetrodotoxin to block Na^{+} channels, and the internal solution in the patch electrode contained 150 mM KCl. The lines through the current traces are the fit to a single exponential function with time constants of 40-50 ms. The activation curve is shown in B. Part C shows the voltage dependence of the time constant of current decay (top) and the current-voltage relation for the peak current (bottom).

Design an experiment <u>using only voltage clamp steps</u> that would reveal whether the decline in the amplitude of the whole cell current during the test pulse is due to (a) inactivation of the calcium current or (b) activation of a calcium-dependent potassium current superimposed on a non-activating calcium current. Draw the current records that you would expect for the two possibilities.



Differences in concentration of ions on opposite sides of a cellular membrane lead to a voltage called the membrane potential. Typical values of membrane potential are in the range –40 mV to –80 mV. Many ions have a concentration gradient across the membrane, including potassium (K^+), which is at a high inside and a low concentration outside the membrane. Sodium (Na^+) and chloride (Cl^-) ions are at high concentrations in the extracellular region, and low concentrations in the intracellular regions. These concentration gradients provide the potential energy to drive the formation of the membrane potential. This voltage is established when the membrane has permeability to one or more ions. In the simplest case, illustrated here, if the membrane is selectively permeable to potassium, these positively charged ions can diffuse down the concentration gradient to the outside of the cell, leaving behind uncompensated negative charges. This separation of charges is what causes the membrane potential. Note that the bulk solutions of either side of the membrane are electro-neutral. Likewise, the system as a whole is electro-neutral. The "uncompensated" positive charges outside the cell, and the uncompensated negative charges inside the cell, physically line up on the membrane surface and attract each other across membrane. Thus, the membrane potential is physically located only in the immediate vicinity of the membrane. It is the separation of these charges across the membrane that is the basis of the membrane voltage. Note also that this diagram is only an approximation of the ionic contributions to the membrane potential. Other ions including sodium, chloride, calcium and others play a more minor role, even though they have strong concentration gradients, because they have more limited permeability than potassium. Key: Blue pentagons - sodium ions; Purple squares - potassium ions; Yellow circles - Choloride ions; Orange rectangles - Anions (these arise from a variety of sources including proteins). The large purple structure with an arrow represents a transmembrane potassium channel and the direction of net potassium movement.

Question #2: 12 minutes (5 points)

The image to the left is taken from the Wikipedia site on "Membrane Potential." The legend to this figure (below the figure) does not have any significant errors. However, the graphic has a couple of items that are at least misleading.

First, most of the charged molecules on either side of the membrane are drawn as salts (where ions of opposite polarity are associated). This is not correct. Most of the ions will be free and hydrated at ~0.15M.

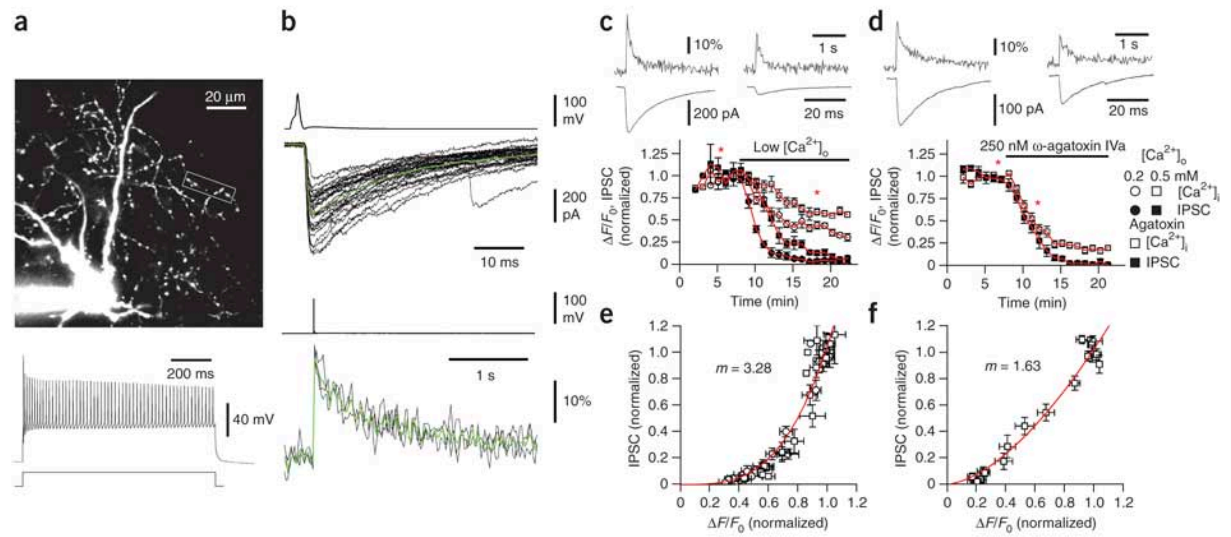
Second, the density of charge on either side of the membrane is not physiological. For illustration purposes, neuroscientists often place many excess charges on each side of the membrane. Let's explore how many is too many.

a) For the figure here, assume that the membrane in question is separating seven monovalent charges and that the area of membrane that does this represents a slab of 25 nm (high) by 4 nm (deep). Calculate the voltage difference across the membrane, given this degree of charge separation. Assume a standard capacitance for biological membranes. (2 points)

b) There is one potassium channel drawn into this figure. Calculate the specific membrane resistance if there is one such channel per 25 x 4 nm area of membrane, if the conductance of one channel is 40 pS, and if the resistance of the rest of the membrane (with no channels) is 10^7 ohm cm^2. (1.5 points)

c) What would be the input resistance of a typical cell (diameter: 20 μm) with the specific membrane resistance you calculate for part **c**? (assume that the cell is spherical) (1.5 points)

Question #3: 15 minutes (7 points)



(**a**) Confocal stack projection of a basket cell filled with 200 µM Oregon green BAPTA 1 and 100 µM Alexa Fluor 594. Box, region of interest used for presynaptic Ca^{2+} measurements. Bottom, fast-spiking action-potential phenotype of the basket cell. Upper trace, voltage. Lower trace, current (1 s, 700 pA). (**b**) Paired recording combined with presynaptic Ca^{2+} imaging. Upper panel, presynaptic action potentials in the basket cell (top traces) and simultaneously recorded IPSCs in the granule cell (bottom traces). 30 consecutive events are superimposed with the average (green curve). Lower panel, corresponding average Ca^{2+} transients in putative boutons near the recorded granule cell (bottom traces), together with presynaptic action potentials (top traces). Three averages of ten individual Ca^{2+} transients each are superimposed with the grand average (green curve). Data in **a** and **b** are from the same pair of neurons. (**c**,**d**) Plot of peak amplitudes of IPSCs (filled symbols) and $\Delta F/F_0$ (open symbols) evoked by single action potentials against time during reduction of extracellular Ca^{2+} concentration from 2 mM to either 0.2 mM or 0.5 mM (**c**, circles and squares, five and seven pairs, respectively) and application of 250 nM ω-agatoxin IVa (**d**, squares, 12 pairs). Each point represents a measurement from the average of seven consecutive sweeps. Red lines connect data points. Both IPSCs and $\Delta F/F_0$ were normalized to values in a 3–8-min control period for each experiment. Traces of average Ca^{2+} transients and corresponding IPSCs from a representative pair are shown on top (averages of 14–35 single sweeps) at the times indicated by asterisks. (**e**,**f**) The relationship between IPSC and $\Delta F/F_0$ during reduction of extracellular Ca^{2+} concentration from 2 mM to either 0.2 or 0.5 mM (**e**, circles and squares) and application of 250 nM ω-agatoxin IVa (**f**). Red curves, fitted power function. Error bars represent s.e.m.

The figure above was taken from a paper that concluded that very few calcium channels are required to trigger transmitter release at a GABAergic synapse. The key evidence in support of the conclusion is found in this figure.

In **b** (top) is shown 30 superimposed traces in response to an action potential in the presynaptic cell. In **b** (bottom) is shown the results of calcium transients for boutons near the recorded granule cell. These records are for the same 30 responses shown in the top part of **b**, but consist of three traces, each of which is averaged from 10 individual responses.

a) What are the two most important sources of trial-to-trial variability in the current responses in **b**? (1.5 points)

b) The decay time constant of the currents in **b** is considerably slower than the decay of AMPA-mediated synaptic currents and yet faster than the decay of NMDA-mediated currents. What accounts for these differences? (1 point)

c) In part **c** is shown the effects of reducing the extracellular calcium concentration on both the calcium signal and the peak current responses. In **e** is shown the "classic" higher power relationship between calcium and response (a proxy for release). What is the molecular basis for this higher power relationship? (1 point)

d) When the calcium signal is reduced by blocking calcium channels with a high-affinity antagonists (agatoxin, part **d**), the relationship between current and signal is changed: it is less cooperative or more linear (compare **e** and **f**; the variable m would have been 1.0 had the relationship been linear). Why do differences between the findings in **e** and **f** support the principal conclusion of the paper? [As a hint, consider the extreme scenario where the authors found a linear relationship in **f**; consider why the relationship is exponential (depending on the ~4th power) in **d** and linear in **f**.] (3.5 points)

Question #4: 10 minutes (4 points)

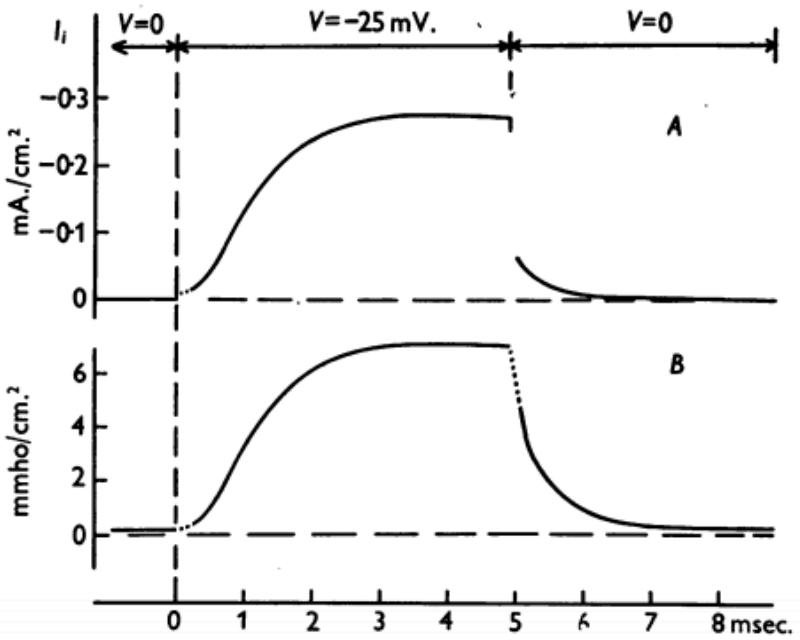


Fig. 13. *A*, ionic current associated with depolarization of 25 mV. lasting 4·9 msec. Axon 18 in choline sea water at a temperature of 21° C. The curve is a direct replot of the original current record except in the regions 0–0·3 msec. and 4·9–5·2 msec., where corrections for capacity current were made by the usual method. Outward current shown upward. *B*, potassium conductance estimated from *A* by the equation $g_K = I_K/(V - V_K)$, where V_K is 12 mV. and I_K is taken as the ionic current (I_i) minus a leakage current of 0·5 m.mho/cm.² × (V + 4 mV.).

The figure above is taken from H&H1952c. This record shows potassium current in response to a step depolarization of 25 mV from rest. The two traces show current (above) and conductance (below).

a) How did H&H eliminate sodium current in this experiment? (0.5 points)

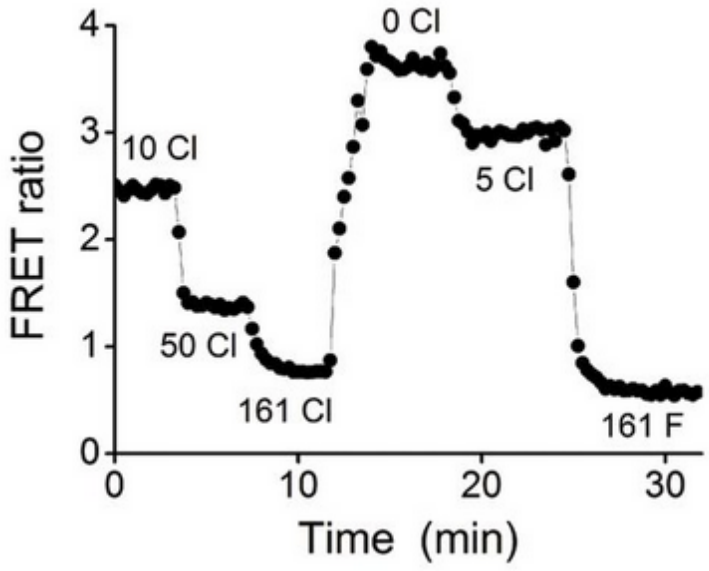
b) How did H&H determine conductance? (0.5 points)

c) What is the cause of the large discontinuity in the current trace at the end of the voltage step? (1.5 points)

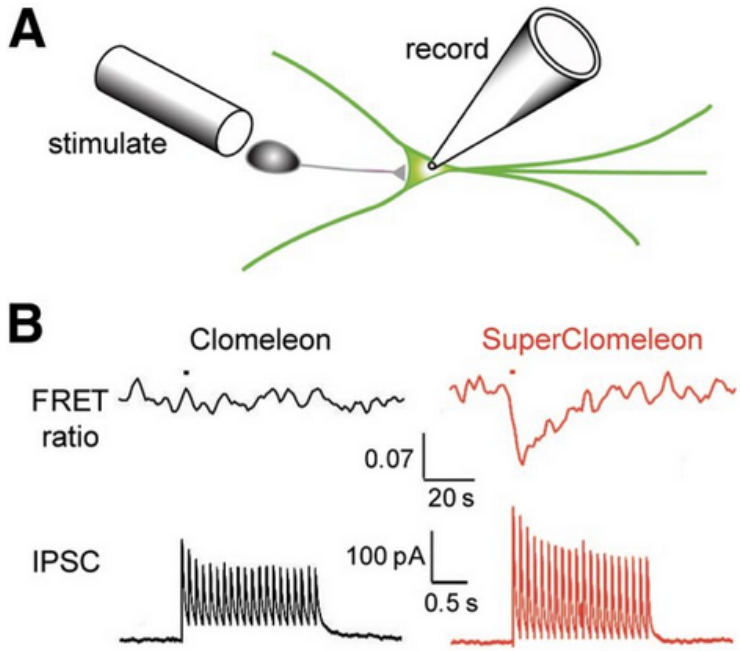
d) Calculate E_K. Assume that the potential at rest is -70 mV. (1.5 points)

Question #5: 20 minutes (8.5 points)

In the figure below, reproduced from a recent J. Neurosci. paper, a fluorescence signal from a synthetic chloride indicator dye (SuperChlomeleon) expressed in a neuron is shown to vary with the internal concentration of chloride. Note that the "FRET ratio" changes by about 1 unit when the internal chloride is changed from 0 to 5 mM $[Cl^-]_i$. (you are going to need this underlined tidbit later!)



In a second figure (below), the authors show that they can measure a change in FRET ratio at a fast time scale, thus illustrating that SuperChlomeleon might be useful as a optical indicator of inhibition (ignore the left side of part B, which shows results with a less sensitive variant of the dye).



Assume the following:

1. The threshold for detecting a change in FRET signal is 0.02 units when$[Cl^-]_i$ is at physiological levels.
2. that in the range of $[Cl^-]_i$ of 0-10 mM, there is a linear relationship between FRET ratio and $[Cl^-]_i$
3. that only one afferent in the slice that terminates on the target cell, and you can stimulate it reliably.
4. that the afferent (above) branches repeatedly before reaching the target cell and ends in many boutons, having many active zones.
5. that release at this synapse is univesicular.

Part A: calculate the change in $[Cl^-]_i$ represented by the minimally detectable level for FRET signal (0.02 units). (1.5 points)

Part B: How many quanta will have to be released at the synapse in order to produce a just detectable change in $[Cl^-]_i$? (5 points)

Assume the following:

1. V_m at rest is -60 mV. E_{Cl} is -45 mV.
2. Fifty $GABA_A$ receptors open per released quantum of transmitter. The $GABA_A$ receptor channels open for an average of 50 ms, and each has an open channel conductance of 50 pS.
3. The volume of the cell is 4×10^{-12} liters.

Part C: In an instance where chloride is passively distributed across the membrane of a neuron and where you are clamping the membrane at resting potential, this fluorescence technique will fail to produce a change in signal, regardless of how much transmitter is released. Why? What might you alter in such instances to produce a signal? (2 points)

Part D: (Extra credit) The time course of the fluorescence signal (B, upper right) is slow relative to the time course of the synaptic current. The signal fails to follow faithfully the responses recorded in response to a train of presynaptic stimuli. Would you expect a chloride indicator dye like SuperChlomeleon to follow faithfully the current trace? Why or why not? (1 point bonus)

Kirichok Section

Question #6 : (15 minutes, 6.75 points)

Why do ions not permeate through the lipid bilayer without special transport proteins? What is the main principle by which ion channels make such permeation possible?

Question #7 : (15 minutes, 6.75 points)

The voltage-sensor domain (VSD) of voltage-gated ion channels consists of 4 transmembrane helixes (S1-S4). Positively charged S4 helix moves within the lipid bilayer in response to changes in the transmembrane voltage and via the S4-S5 linker makes the pore to close and open. Do you think it would be possible to construct a simpler VSD that would consist of S4 domain only? Please explain your answer.

Edwards Section

Question #8 : (15 minutes, 6.75 points)

A knockout mouse shows increased synaptic depression with high frequency stimulation. Using a combination of postsynaptic recording and optical imaging, how can you determine whether this reflects a change in release probability or a defect in recycling?

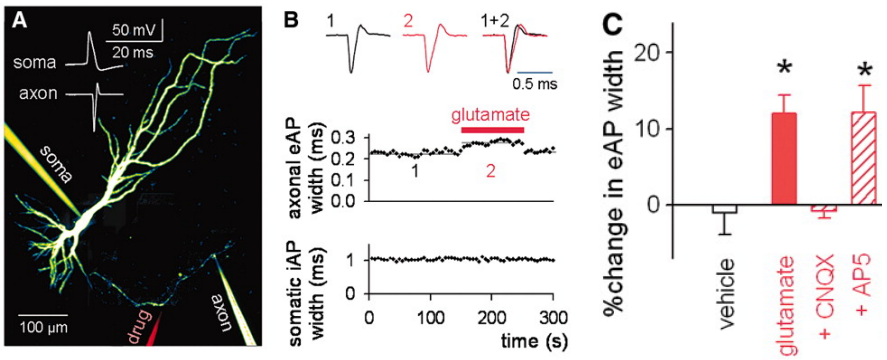
Question #9 : (15 minutes, 6.75 points)

You have just found that heterologous expression of an orphan polytopic membrane protein confers currents in response to the addition of a novel neurotransmitter. How can you distinguish between the mechanism for this charge movement as either a channel (i.e., channel for other ions but gated by the transmitter) or as a transporter? Do not simply look for other assays that would support channel versus transporter activity since the protein may in fact have multiple functions—rather, use the currents themselves to address the mechanism that underlies them.

Bender Section

Question #10 : 18 minutes, 9 points:

In panel "A", two recordings are made from a single hippocampal neuron: one whole-cell current clamp from the soma and one cell-attached voltage-clamp from the axon. A drug is applied via a 3rd pipette to



a region of axon proximal to the cell-attached pipette. An AP is initiated by injecting current through the somatic pipette, and the resultant propagating AP passes through the region where "drug" is delivered and is eventually recorded again by the cell-attached pipette. CNQX and AP5 are AMPA and NMDA receptor antagonists, respectively.

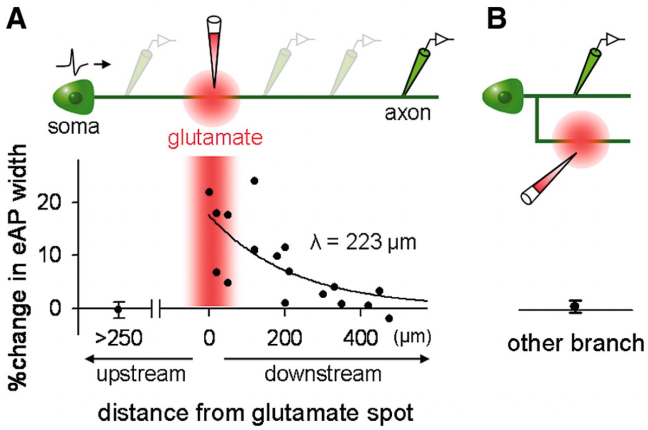
In panel "B" the APs "1" and "2" are not the same width. AP 1 was recorded in control conditions, AP 2 was recorded when glutamate was applied to the axon.

a) What happened to the spikes recorded in the downstream axon after application of glutamate? (0.6 points)

b) Would this change in spike shape alter release probability (increase, decrease, or no change)? Why? (1.2 points)

c) What receptors were activated by glutamate? Justify your answer. (1.2 points)

d) By acting on axonal receptors identified in panel "C", glutamate is likely changing the membrane potential of the axon. Would the axon be depolarized or hyperpolarized? What effect would this have on Na^+ and K^+ channel availability? Propose an experiment to determine whether either of these two channel classes was altered by the glutamate puff. You have access to the same tools as were used above, including dual recording, puffer apparatus, and pharmacology. (3 points).



e) Above, serial cell-attached recordings were made at various distances upstream and downstream of the glutamate puff. AP width was broader downstream, but not upstream, of the glutamate puff. Why was upstream AP width unaltered? (0.6 points)

f) The broadening of AP width has a length constant in the downstream direction. Redraw panel A showing both this baseline effect and draw a new length constant if the cell lacked myelin. Just draw the exponential fits in both cases. (1.2 points)

g) same as question "f", but instead of demyelinating the axon, draw the fits if a $GABA_A$ hotspot were located 200 microns downstream of the glutamate puff. Assume that GABA is shunting and tonically active. (1.2 points)

Question #11 : 12 minutes, 4.5 points:

Suppose you've made a single whole-cell, current-clamp recording from the apical dendrite of a neocortical layer 5 pyramidal cell in an "active" slice preparation. In this slice preparation, the local network of pyramidal cells can be spontaneously active (i.e., both the cell from which you are recording and neighboring cells *may* be firing spikes). You observe electrical events from your dendritic recording site that look like EPSPs, but you now know that back propagating action potentials can be filtered as they back propagate from the soma to your recording site, and can start to look more like EPSPs than APs when recording in the dendrite.

Propose a set of experiments to establish convincingly whether these events are generated by local tuft synapses or by back propagating APs. Explain how your experiment will alter the frequency and/or amplitude of these events if they were mediated by EPSPs or bAPs. If you can envision pitfalls to your approach, discuss them.

If you so choose, you may apply pharmacological agents to the entire slice. Unfortunately, you spilled beer on your second manipulator, so you can't patch the cell with a second electrode. Nor can you apply drugs to just one small region of the cell. Further, there's beer in your laser, so you can't image the neuron. You really need to get a spill proof mug.

Bonus half point: How, in one sentence, you're going to explain the beer incident to your PI.

Whistler Section

Question #13 : 4 minutes, 1.75 points

Extended True or false: you have two neutral antagonists at the same target with the same affinities. Both drugs have the same potency. If true, why. If false, why not.

Question #14 : 11 minutes, 5 points

In the striatum, cocaine causes an increase in dopamine tone by blocking the dopamine transporter. D2 dopamine receptors, which are G_i coupled, are located on the presynaptic terminals of the dopamine neurons, where they serve as autoreceptors inhibiting transmitter release when activated. Both D2 and D1 (G_s coupled) receptors are located postsynaptically. After endocytosis in response to dopamine, D2Rs are downregulated while D1Rs are not.

a) How would tonic dopamine release in the striatum be altered in cocaine treated animals assuming that the cocaine pretreatment caused no change in D2 receptor affinity for dopamine?

b) What would be the predicted effect of cocaine pretreatment on tonic dopamine release assuming that cocaine pretreatment shifted more D2 receptors into the G protein coupled state?

Question #15 : 11 minutes, 5 points

You are screening for antagonists of Your Favorite Receptor YFR using a functional assay in which YFR is expressed in heterologous cells. You identify two molecules, A and B, both of which block activation of YFR in response to your selective agonist compound for YFR with the same potency. Neither has any effect on receptor activity on its own. You then use competition binding with a radioligand antagonist to determine the affinity of A and B. A has high affinity. However B has very low affinity.

What property of B allows it to be so potent despite its low affinity?

Question #16 : 4 minutes, 1.75 points

Extended True or false: you have two agonists at the same target with the same potencies. Both drugs must have the same affinity. If true, why. If false, why not.

Nicoll section

Question #17 : 30 minutes, 13.5 points.

The recent discovery that exogenously expressed kainate receptors can undergo NMDA-dependent LTP is most provocative because these receptors are quite distinct from AMPA receptors. This finding has resurrected the presynaptic mechanism for LTP whereby the increase in glutamate release could account for the enhanced kainate receptor mediated responses. Is this finding sufficient grounds for returning to a presynaptic expression mechanism for NMDA-dependent LTP? Please discuss.